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CHEMORECEPTION IN THE SHRIMP, *PALAEMONETES PUGIO*: COMPARATIVE STUDY OF STIMULATORY SUBSTANCES IN HUMAN SERUM[1]

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There is evidence that several aspects of the behavior of marine crustaceans are influenced or directed by external chemical agents. Studies with various crustaceans have indicated that external chemical agents are involved with such diverse phenomena as feeding behavior (Case and Gwilliam, 1961; Crisp, 1967; Laverack, 1963; and others), host location by commensals (Ache and Case, 1969; Davenport, 1966), mate recognition (Kittredge, Terry and Takahashi, 1971; Ryan, 1966), and prey concealment (Kittredge, Takahashi, Lindsey and Lasker, 1974). Chemical stimulation of feeding behavior has received the attention of many investigators (for review, see Lindstedt, 1971). Effectively all of the studies on this phenomenon in marine crustaceans have focused on the stimulatory capacity of substances of low molecular weight, especially amino acids and amines. Inadequate attention has been given to whether or not the substances studied actually occurred in sufficient concentrations in potent preparations such as extracts or body fluids to account for the responses given by the experimental animal to the latter preparations.

The report that follows has a twofold purpose: 1) to describe a procedure for studying a chemically mediated feeding response in the shrimp, *Palaemonetes pugio*, and 2) to account for the response-inducing capacity of human serum in terms of the compounds present and their relative concentrations in serum. Human serum was selected as the standard for this initial study with *P. pugio* because in addition to being a response-inducer, a large literature exists on its chemical composition and many of its principal components are available commercially. This report also includes some preliminary results concerning the nature of the major stimulants present in certain other body fluids and extracts.

Methods

Maintenance of animals

Groups of several hundred *Palaemonetes pugio* were collected every two weeks with a dip net near the Whitney Marine Laboratory. Each group was maintained in a 35-gal aquarium provided with running filtered sea water, aeration, and a thin layer of fragmented beach shell on the bottom. An acclimation period of 14 to 18 days in the laboratory was required before each new group could be used in experiments. During the acclimation period the group was fed daily with

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TetraMin (Tetra Sales Corp., Hayward, Calif.). After the initiation of experiments the group was fed once a week. Another source of food consisted of recently molted individuals which were cannibalized regularly. Following the acclimation period, experiments with a group of shrimp continued over a two-week period after which the group was replaced. Individual shrimp were used in no more than one bioassay per day.

Bioassay procedures

Bioassays were conducted in 27 × 17.5 × 4 cm plastic boxes (Plano Molding Co., Plano, Ill.) divided by partitions into six compartments of equal size. Each box was placed inside a wooden frame over which a plexiglass sheet was mounted. Solutions to be tested were introduced into each compartment through a Bubble Cone aerator (Marineland Aquarium Products, Aquaria, Inc., Los Angeles). The Bubble Cone (referred to hereafter as the "target") was affixed to a section of 3-mm Tygon tubing and entered each compartment through a hole (16-mm dia) in the top of the box. Holes (13-mm dia) in the plexiglass sheet above each box received a No. 00 rubber stopper bored to accommodate the Tygon tubing. This arrangement permitted the target to be introduced into each compartment with minimal disturbance to the shrimp and to be secured in a stationary position with the tip approximately 1 cm from the bottom of the box. Shrimp could move freely beneath the tip of the target. A 5-ml pipette affixed to the Tygon tubing and held in place by a burette clamp served as the reservoir for each solution to be introduced through the target. Flow rate was regulated with a screw clamp and stopped or started with a pinch clamp. The pipette was refilled after each test by suction with a rubber bulb.

Bioassays were standardized as follows. (1) Each plastic box was prerinsed in sea water and to each compartment was added 100 ml of water from the aquarium containing the shrimp to be used. Three shrimp were transferred by dip net into each compartment to give a total of 18 shrimp per box (one test group). (2) Each box of shrimp was placed in its wooden frame and left undisturbed for 60 minutes. When a solution was to be tested at several concentrations, several boxes of shrimp were prepared as above at 25 minute intervals so that each successive box remained undisturbed for 60 minutes and was ready for testing shortly after completing tests with the preceding box. (3) A single concentration of a solution was used for each entire box of shrimp. The solution was introduced into each compartment for a 1.5 minute period at a flow rate calibrated to deliver 3.6 to 4.0 ml during this period. The response of shrimp to stimulatory solutions is described in the results. The pipette, tubing and target were rinsed thoroughly in deionized water and filtered sea water before and after each concentration of a solution was tested. All solutions were prepared in the same filtered sea water that was provided to the aquarium maintaining the shrimp. During bioassays, compartments containing injured or newly molted shrimp were not tested.

Preparation of solutions for bioassay

For testing single compounds or mixtures of compounds, a concentrated stock solution was prepared in filtered sea water and kept in ice. When necessary the

pH was adjusted to 7 to 8 as determined with Alkacid paper. A solution of the desired concentration was prepared by pipetting a small volume of stock solution into the appropriate volume of sea water.

Fresh frozen human serum was obtained from the Clinical Chemistry Laboratory, J. Hillis Miller Health Center, University of Florida. Lyophilized human serum, human alpha$_1$-globulins (Fraction IV-1), beta globulins (Fraction III), gamma-globulins (Fraction II), glycoprotein (orosomucoid, Fraction VI), and albumin were obtained from Miles Laboratories. Purified plasma lipoproteins were prepared by Dr. Richard Triplett, Department of Medicine and Biochemistry, University of Florida.

Ultrafiltration and dialysis

Ultrafiltration through Amicon UM-2 membranes retaining molecules greater than *ca.* 1000 molecular weight was carried out in a 50-ml stirred cell with 30 psi of N_2 at 4° C. Certain protein solutions were prepared in 1% NaCl and dialyzed for 24 hr at 4° C with constant stirring in 20-mm Visking tubing. Dialysis was effected by four changes of one liter of 1% NaCl.

Preparation of body fluids and extract from other sources

All preparations were made from live specimens. Hemolymph serum of male blue crabs (*Callinectes sapidus*), extract of pink shrimp (*Penaeus duorarum*) and oyster fluid (*Crassostrea virginica*) were prepared as described by Carr, Hall and Gurin (1974) and by Gurin and Carr (1971). Extract of spiny lobster (*Panulirus argus*) was prepared by cutting up abdominal muscle in cold deionized water and shaking for 30 minutes in an ice bath followed by centrifugation at 4° C, decantation, and storage in ice. Extract of coquina, *Donax variabilis*, was prepared by grinding up entire animals with a mortar and pestle in cold filtered sea water followed by centrifugation and treatment as above.

Statistical treatment of data and presentation of results

Many of the data which follow were analyzed by Potency Probit Analysis employing the computer program of Daum and Givens (1963) at the Northeast Regional Data Center, University of Florida. A brief description of the program is provided by Carr (1967). Comparisons of the relative potencies of solutions were obtained by assaying solutions over a range of concentrations and determining the concentration at which each solution induced a positive response in 50 per cent of the test animals (effective dose for 50 per cent of test animals = ED_{50}). Unless otherwise indicated each determination of an ED_{50} involved bioassays of at least three concentrations with a minimum of 18 shrimp employed at each concentration. Statements of statistical significance are based on 95% limits of confidence.

In the literature, concentrations of many constituents of human blood are usually expressed in terms of their concentrations in blood plasma. In the current study the concentrations of constituents that are assumed for human serum were obtained from reported concentrations in plasma. Serum is considered to be plasma minus fibrinogen and certain other clotting agents. To compare directly

the potency of certain constituents of human serum with that of serum itself, the concentrations of the constituents are expressed in terms of serum milliequivalents. One serum milliequivalent is defined here as the amount of the particular constituent that would be present per ml of sea water when serum itself was diluted to a concentration of one μl per ml of sea water. Hence, one thousand milliequivalents equals the amount of the particular constituent that would be present in full strength serum (*i.e.*, 1000 μl/ml).

Results

In an aquarium individuals of *Palaemonetes pugio* remained for the most part on the bottom in a reasonably stationary position. However the introduction of pieces of fish, shrimp or other potential food items quickly stimulated a marked change in behavior. The initial recognition of the presence of food was indicated by increased movement followed by a rapid swarming around and a distinct searching behavior that included swimming up and down throughout the water column. Food itself was not required for the stimulation of such behavior since the addition of small amounts of filtered extracts would induce the same response. Likewise, small pieces of sponge soaked in an extract would be sought out and converged upon by groups of shrimp. Pieces of clean sponge soaked only in sea water were ineffective. These preliminary observations led to the development of the bioassay procedure used in the current study.

In the plastic boxes used for bioassays, the introduction of a strong stimulant is quickly detected by individuals in a compartment and the target is usually found within a few seconds. Preliminary tests with several hundred individuals over various periods of time revealed that a standardized test period of 1.5 minutes was adequate for obtaining responses from effectively all individuals that would respond at all. A positive response in the bioassay procedure consists of a shrimp moving to the target and grasping it. Since the lower tip of the target was *ca.* one cm from the bottom of the compartment, each positive response required that the shrimp leave the bottom and suspend himself near the surface when the target is grasped. This behavior was very seldom observed in the absence of stimulant. In controls that were run during the course of these experiments, only two individuals out of 440 (0.5%) gave positive responses when filtered sea water alone was introduced through the target.

Tests with glycine

During the initial stages of this investigation, it was noted that glycine was a response inducer. Hence glycine was used to determine the degree of reliability of the procedure. Fresh glycine solutions were tested repetitively on four separate days with individuals from a single group of shrimp and with individuals from four separate groups collected at different times during a period of three months. With individuals from the same group of shrimp, the ED_{50} of glycine averaged 3.7×10^{-3} M and ranged from 2.4×10^{-3} to 5.2×10^{-3} M. These determinations were not significantly different. With individuals from separate groups the ED_{50} of glycine averaged 4.9×10^{-3} M and ranged from 2.8×10^{-3} to

7.2×10^{-3} M. These determinations were not significantly different. On the basis of these repeated assays of glycine it was evident that the procedures had a satisfactory degree of reliability and could be used to measure the relative potency of solutions. The decision to use a 14 to 18 day period of acclimation prior to the initiation of experiments with a new group of shrimp (see methods) evolved primarily from the above studies with glycine. Shrimp kept in the lab for shorter periods did not adjust well to confinement in the plastic boxes. Many would continue to scurry about in the compartments and jump against the walls even after a one-hour period. Shrimp in this hyperactive condition would frequently even refuse to accept food and could not be tested. However, after 14 to 18 days in captivity this hyperactive response to transfer and confinement decreased dramatically and by the end of a one-hour period effectively all individuals had assumed a reasonably inactive state.

TABLE I

Concentrations of substances in human plasma and responses of P. pugio *to these substances.*

Substance(s)	Upper limit of normal conc. in human plasma (mg/ml of plasma)	Concentrations tested		Per cent of shrimp responding
		mg/ml	serum milliequivalents per ml	
Albumin	45†	1.0	22	22
		5.0	110	28
Alpha$_1$-globulins	6†	0.12	20	11
		0.6	100	50
		1.5	250	56
Orosomucoid	0.75†	0.075	100	7
		0.375	500	33
Beta-globulins	11†	0.11	10	17
		1.1	100	11
Gamma-globulins*	15†	0.15	10	6
		0.6	40	17
		1.2	80	33
High density lipoprotein	5.3‡	0.33	60	13
		0.66	120	17
Low density lipoprotein	4‡	0.14	35	27
		0.28	70	53
		0.56	140	56
Very low density lipoprotein	2‡	0.04	20	13
		0.4	200	50
Glycogen	0.06†	0.1	1700	6
		1.0	17000	56

† Concentrations derived from White, Handler and Smith (1964, pp. 628 and 633). Values for proteins presented by White *et al.* are in units of g/100 ml.

‡ Concentrations derived from Scanu and Wisdom (1972).

* Gamma-globulins dialyzed exhaustively for 24 hours prior to testing to remove glycine added by Miles Laboratories as a stabilizing agent.

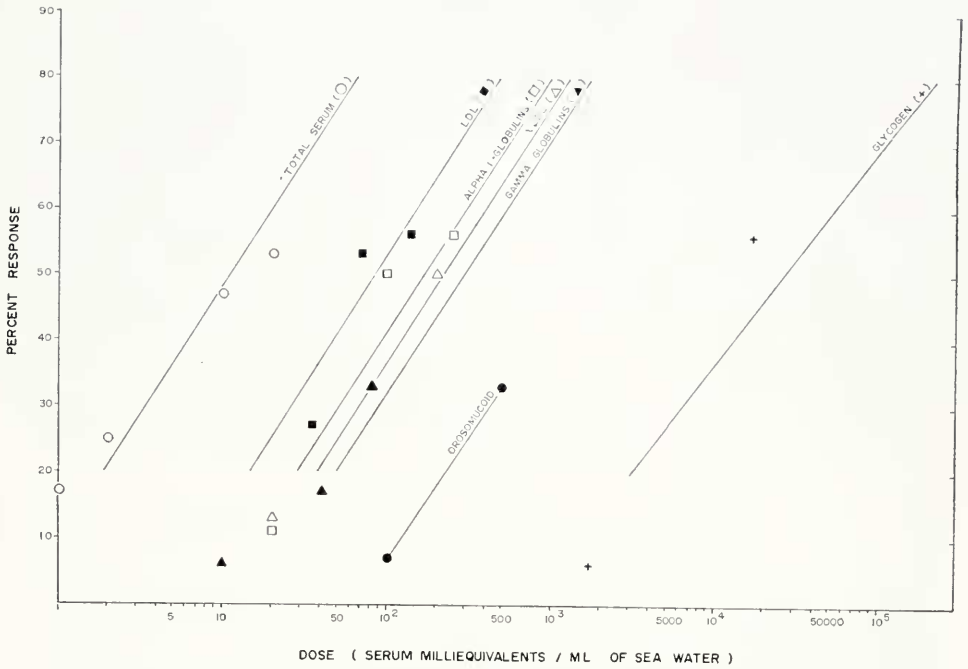

FIGURE 1. Responses given by *P. pugio* to human serum and to certain serum proteins and glycogen. Regression lines were drawn from computed effective dose values obtained by Potency Probit Analysis. The potency (and 95% confidence limits) of low density lipoprotein (LDL) and alpha$_1$-globulins were 0.131 (0.444–0.049) and 0.065 (0.197–0.024) respectively. Potency × 100 equals percent of activity of total serum.

Studies of stimulatory substances in human serum

Following ultrafiltration of human serum through a UM-2 membrane it was found that the retentate containing substances of greater than *ca.* 1000 MW was as active as the total serum. The ultrafiltrate containing smaller molecules was significantly less active and accounted for less than 20 per cent of the activity of the total serum.

In human serum, proteins and glycogen are the major substances with molecular weights greater than 1000. Table I contains the results of bioassays of many of these substances together with the normal concentrations at which they occur in human plasma. All of the substances possessed a certain degree of response-inducing activity. When the concentrations tested are considered solely on the basis of mg per ml of sea water, the activities of five of the substances (alpha$_1$-globulins, orosomucoid, low density lipoprotein, very low density lipoprotein, and glycogen) are quite similar. The ED_{50} values of these five substances were estimated to range between *ca.* 0.3 and 0.8 mg per ml of sea water. Values for the other substances were considerably higher. Since there are, however, differences in the inherent concentrations of these various substances in serum itself, these differences must be considered in assessing the relative contribution that each sub-

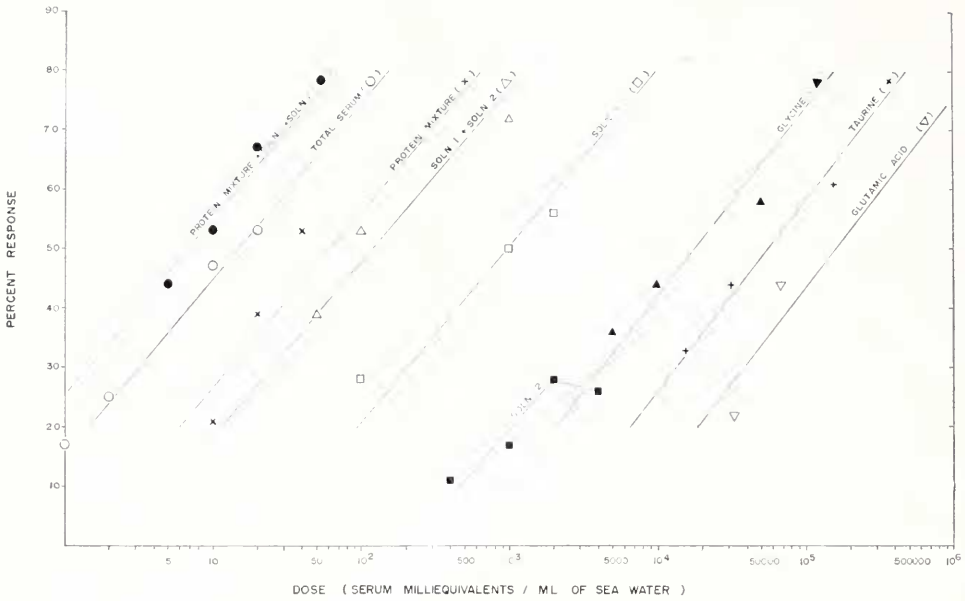

FIGURE 2. Responses given by *P. pugio* to human serum and to certain serum components and mixtures of components. Regression lines were drawn from computed effective dose values obtained by Potency Probit Analysis. No regression line was computed for results obtained from Solution 2. Potencies (and 95% confidence limits) of major mixtures were as follows: Protein mixture + Solution 1 + Solution 2 = 2.226 (15.311–0.659); Protein mixture = 0.239 (0.875–0.066); Solution 1 + Solution 2 = 0.127 (0.874–0.035); Solution 1 = 0.016 (0.073–0.005). Potency × 100 equals percent of activity of total serum.

stance(s) might make to the potency of serum. Differences in inherent concentrations are illustrated in Figure 1 which expresses all concentrations in terms of serum milliequivalents (see methods). The figure illustrates the potency of certain of these serum components relative to the potency of serum itself. Individually, each type of protein (and glycogen) could account for only a small portion of the potency of total serum. Low density lipoprotein and the alpha$_1$-globulins were the most potent of these substances and accounted for approximately 13 per cent and 7 per cent of the potency of serum respectively. The other substances, though all possessing various degrees of activity could, individually, account for less than 5 per cent of the potency of total serum.

The finding that none of the separate groups of proteins (or glycogen) cited above was able to account for a major part of the potency of total serum suggested that the potency of serum might be attributed to a mixture of two or more of these groups. A mixture of human albumin, alpha$_1$-globulins, beta globulins, gamma-globulins, low density lipoprotein and orosomucoid was prepared such that each constituent was present at the same relative concentration as in serum. This mixture was dialyzed exhaustively and bioassayed. Figure 2 shows that this mixture was somewhat more active than any of its individual components and could account for approximately 24 per cent of the potency of the total serum.

Table II gives the composition of stock solutions that were prepared for measuring the potency of mixtures of the 37 major organic constituents of low molecular weight found in human plasma. For convenience these substances were divided into two groups for testing: Solution 1—comprised of amino acids, taurine, and urea; Solution 2—comprised of other small nitrogenous substances, carbohydrates, and organic acids. Figure 2 shows that the mixture of substances in Solution 1 could account for only approximately two per cent of the potency of serum whereas the components of Solution 2 were even less active. A mixture of substances in both solutions (Solutions 1 and 2) was considerably more effective but could account for only approximately 13 per cent of the potency of total serum. The potency of the latter mixture compared favorably with the potency of the UM-2 ultrafiltrate described earlier.

Of the substances tested thus far in this study, the proteins made the largest contribution to the potency of total serum. However, the failure of the mixture of known proteins to account for the entire potency of serum suggested that the

TABLE II

*Composition of solutions prepared for measuring potency of substances of low molecular weight found in human plasma.**

Solution 1		Solution 2	
Compounds included	Upper limit of normal conc. in human plasma (mg/100 ml)	Compounds included	Upper limit of normal conc. in human plasma (mg/100 ml)
Alanine	3.7	Bilirubin	1.4
Alpha-aminobutyric acid	0.4	Citric acid	3.0
Arginine	1.9	Creatine	0.9
Asparagine	0.7	Creatinine	2.0
Aspartic acid	0.07	Fructose	8.0
Cysteine and cystine	1.3	Glucose	90.0
Glutamic acid	1.2	Alpha-ketoglutaric acid	1.0
Glutamine	12.0	Lactic acid	17.0
Glycine	1.7	Malic acid	0.9
Histidine	1.5	Ornithine	0.8
Isoleucine	1.3	Pyruvic acid	2.0
Leucine	2.3	Succinic acid	0.6
Lysine	3.0	Uric acid	6.0
Methionine	0.4		
Phenylalanine	1.0		
Proline	3.3		
Serine	1.2		
Taurine	0.8		
Threonine	1.7		
Tryptophan	1.2		
Tyrosine	1.5		
Urea	30.0		
Valine	3.7		

* Compounds and concentrations given by White, Handler and Smith (1964, p. 628). Stock solutions were prepared with all constituents present at concentrations ten-fold the values shown in table.

presence of certain substances of low molecular weight, together with the proteins, might be necessary to account for the total activity of serum itself. To test this possibility a mixture containing the six dialyzed proteins described earlier was combined with a mixture of Solution 1 and Solution 2 for testing. Figure 2 shows that this mixture provided a solution that was fully as active as total serum. The fact that this mixture was seemingly somewhat more potent than serum itself was probably due to the fact that each substance in the mixture was added at a concentration proportionate to the normal upper limit reported for plasma. Although this practice provided solutions in which the various constituents of serum occurred at their proper relative concentrations, this practice logically resulted in the incorporation of certain constituents at concentrations higher than those which actually existed in the samples of total serum that were used as a standard.

Bioassays of individual substances of low molecular weight

In conjunction with the tests of mixtures of substances of low molecular weight found in human serum, several of these same substances were assayed individually over a range of concentrations of 10^{-2} to 10^{-4} M. In addition to glycine (ED_{50} *ca.* 5×10^{-3} M), two other substances were found to be moderately strong response-inducers when tested alone. These substances were L-glutamic acid (ED_{50} *ca.* 10^{-2} M) and taurine (ED_{50} *ca.* 4×10^{-3} M). Individually, the activities of glycine, glutamic acid, and taurine accounted for only 0.01 to 0.08 per cent of the potency of total serum (see Fig. 2). Slight activity was also obtained with citric acid, D-glucose, and betaine (not reported to be a constituent of human plasma). However, the ED_{50}, if any, for each of these latter substances was greater than 10^{-2} M. A mixture of glycine, glutamic acid, taurine, citric acid, and glucose was prepared with each substance present at the same relative concentration as in plasma (see Table II). This mixture could account for only approximately one per cent of the potency of serum itself and was slightly less active than the mixture of substances in Solution 1 described earlier.

None of the following substances were response-inducers over the range of concentrations indicated as follows: 10^{-2} to 10^{-3} M, N-acetylglucosamine, N-acetylglycine, glycerol, lactic acid, proline, and trimethylamine oxide; 10^{-3} to 10^{-4} M, glutathione, glycerophosphorylcholine, octopine, octopinic acid; 10^{-4} to 10^{-5} M, cyclic adenosine monophosphate, L-3,4 dihydroxyphenylalanine (DOPA), L-epinephrine, melatonin, and serotonin. Of the substances listed above, only lactic acid and proline are considered to be among the major constituents of normal human plasma.

Preliminary tests with body fluids and extracts from other sources

A series of other body fluids and extracts were assayed before and after fractionation by ultrafiltration. In two of the preparations substances of greater than *ca.* 1000 molecular weight (presumably proteins) were the major stimulants, whereas smaller molecules were the major stimulants in the other three preparations. With oyster mantle fluid and with coquina extract, the retentates were fully as active as the original preparations. The ultrafiltrates of both were significantly less active. With crab serum, lobster extract and shrimp extract, the

major stimulants were present in the ultrafiltrates which in each case were not significantly less active than the original preparations. The retentates of each of these latter preparations were significantly less active.

Discussion

Palaemonetes pugio proved to be an excellent test animal for studies of chemoreception. This small shrimp abounds near the shoreline of the estuarine habitat near our laboratory. It is easy to maintain in dense populations with the reservation that considerable cannibalism occurs especially upon freshly molted individuals. The bioassay procedure that was described is both convenient and reliable. This procedure allows the investigator to quickly recognize response-inducing solutions and to compare in a quantitative manner the relative potencies of closely related solutions.

Our results clearly show that the effectiveness of human serum as a chemosensory stimulant in *P. pugio* is due to a mixture of substances rather than to a single major stimulant. This mixture of substances includes both proteins and a group of components of low molecular weight. Our finding that proteins make a major contribution to the potency of this mixture provides an important corollary to several other recent reports on the nature of feeding stimulants in other groups of marine invertebrates. Gurin and Carr (1971) showed that in the gastropod, *Nassarius obsoletus* (phylum Mollusca), stimulation of the probosis search reaction by human serum and oyster fluid could be ascribed primarily to very low concentrations of specific proteins. In human serum the major stimulant was serum albumin whereas in oyster fluid it was a large glycoprotein. Additional evidence that proteins present in other biological fluids and extracts serve as important feeding stimulants in *N. obsoletus* was provided by Carr, Hall and Gurin (1974). Mangum and Cox (1971) reported evidence that a glycoprotein in a mollusk extract made a significant contribution to stimulation of a feeding response in the polychaete, *Diopatra cuprea* (phylum Annelida). Heeb (1973) found that protein fractions isolated from two mollusk extracts made an important contribution to stimulation of feeding behavior in the starfish, *Asterias forbesi* (phylum Echinodermata).

In *P. pugio*, the fact that proteins make an important contribution to the stimulatory capacity of human serum does not justify the assumption that proteins make an important contribution to the activity of all preparations that stimulate feeding behavior in this animal. Preliminary tests with other body fluids and with extracts showed that the nature of the major feeding stimulants are apt to vary in preparations obtained from different sources. In crab serum and in extracts of shrimp and lobster it is clear that the major stimulants are not proteins but are substances of reasonably low molecular weight. However, the results of our tests with the common amino acids, amines, organic acids, and carbohydrates suggest strongly that these substances alone will not prove to be the major stimulants since the concentrations at which they occur in these latter preparations are inadequate to account for the potencies of the crab serum or the extracts themselves. Additional studies of the stimulants in these preparations are in progress.

The possession of chemoreceptors sensitive to amino acids and/or other nitrogenous compounds of low molecular weight is characteristic of effectively all

marine arthropods that have been studied to date using either behavioral procedures or electrophysiological techniques. In the current study with *P. pugio,* taurine (*ca.* 0.004 M), glycine (0.005 M), L-glutamic acid (*ca.* 0.01 M), and betaine (> 0.01 M) were found to be response-inducers when tested alone. It is of interest that sufficient concentrations of one or more of the above substances have also been reported to induce feeding behavior and/or elicit chemoreceptor activity in a variety of other marine arthropods. Case and Gwilliam (1961) found that dactyl receptors of the crab, *Carcinides maenas,* were especially sensitive to L-glutamic acid at concentrations as low as 0.00005 M. Moreover, the application of either 0.001 M glutamic acid or of *Mytilus* extract to the chelae stimulated the beginning of feeding movements. However, no report of the concentration of glutamic acid in the extract was given. Laverack (1963) found dactyl receptors of *C. maenas* sensitive to betaine (0.1 to 0.001 M). Laverack (1964) later reported the presence of antennular receptors of the spiny lobster, *Panulirus argus,* that were sensitive to 0.1 M betaine, glycine, and L-glutamic acid. Levandowski and Hodgson (1965) also reported that antennular and dactyl receptors of *P. argus* were sensitive to betaine and L-glutamic acid but less sensitive to glycine. The horseshoe crab, *Limulus,* possesses gnathobase receptors sensitive to 0.5 M glycine, glutamic acid and taurine (Barber and Hayes, 1963). However, clam extracts were noted by the latter workers to elicit responses stronger than any given to amino acids when tested either alone or as mixtures. Case (1964) studied thoroughly certain properties of dactyl chemoreceptors of two crabs, *Cancer antennarius* and *C. productus.* Response magnitudes to various substances were compared to those evoked by 0.05 M glycine. Included among the substances with response magnitudes greater than glycine were taurine, L-glutamic acid and betaine. Crisp (1967) reported that L-glutamic acid and taurine (*ca.* 0.00002 M) were the most effective substances he tested for inducing feeding movements of cirri in two species of stalked barnacles. Betaine (*ca.* 0.0002 M) and glycine (*ca.* 0.002 M) were also active. Two species of commensal shrimp of the genus *Betaeus* were found by Ache and Case (1969) to possess antennular receptors sensitive to glutamate and glycine (*ca.* 0.1 M). McLeese (1970) found glutamic acid (*ca.* 0.7 ppm) to be among the most effective inducers of a feeding response in the lobster, *Homarus americanus.* McLeese pointed out that although several amino acids and other substances induced feeding behavior either alone or in mixtures, none of the substances or mixtures were as stimulatory as extracts of cod, shrimp or lobster muscle. In summary one must conclude that although an array of marine arthropods have been shown to have chemoreceptors capable of detecting certain amino acids and related substances, no studies have shown that these substances can account for the major portion of the stimulatory activity of a single tissue extract or biological fluid.

Copeland (1923) reported that *Palaemonetes vulgaris* could find fish meat buried in sand or in a tube covered with cloth. His early studies showed that chemical agents emanating from food served to attract the shrimp to this food. The age-old practices of attracting crabs and lobsters into baited traps and of chumming the water for shrimp are examples of the potential that exists for exploiting the chemical sense of marine invertebrates as a means of increasing the harvest of seafood. Technological advances in this area are likely to be forthcom-

ing as soon as the chemical nature of the principal attractants in "baits" and "chum" are determined. Such determinations should be enhanced greatly by the development and utilization of quantitative bioassay procedures which permit the investigator to compare directly the potencies of various baits as well as the potencies of the specific chemical substances that they contain.

Summary

1. A bioassay procedure is described for studying a chemically mediated feeding response in the shrimp, *Palaemonetes pugio*. The procedure involves the attraction of shrimp to a small target in compartmented plastic boxes.

2. Bioassays of purified components of human serum showed that the response of shrimp to serum is due to a mixture of substances including both proteins and substances of low molecular weight.

3. A dialyzed mixture containing six types of serum proteins accounted for approximately one-fourth of the potency of serum. Although all of the proteins possessed a certain degree of activity, low density lipoprotein and the alpha$_1$-globulins were individually the most active components of the mixture.

4. A mixture containing the 37 major low molecular weight organic constituents of serum accounted for only approximately one-eighth of the potency of serum. Glycine, taurine and glutamic acid were the most active constituents of this mixture that were tested individually.

5. A mixture containing the six types of serum proteins together with the 37 low molecular weight constituents was fully as active as the total serum.

6. Ultrafiltration of body fluids or extracts from coquina, crab, lobster, oyster and shrimp showed that in some preparations the major stimulants were large molecules (greater than *ca.* 1000 MW) whereas in others they were small molecules.

LITERATURE CITED

Ache, B., and J. Case, 1969. An analysis of antennular chemoreception in two commensal shrimps of the genus *Betaeus*. *Physiol. Zool.*, **42**: 361–371.

Barber, S. B., and W. P. Hayes, 1963. Properties of *Limulus* chemoreceptors. *Proc. XVI Int. Congr. Zool.*, **3**: 76–78.

Carr, W. E. S., 1967. Chemoreception in the mud snail, *Nassarius obsoletus*. I. Properties of stimulatory substances extracted from shrimp. *Biol. Bull.*, **133**: 90–105.

Carr, W. E. S., E. R. Hall, and S. Gurin, 1974. Chemoreception and the role of proteins: a comparative study. *Comp. Biochem. Physiol.*, **47A**: 559–566.

Case, J., and G. F. Gwilliam, 1961. Amino acid sensitivity of the dactyl chemoreceptors of *Carcinides maenas*. *Biol. Bull.*, **121**: 449–455.

Case, J., 1964. Properties of dactyl chemoreceptors of *Cancer antennarius* Stimpson and *C. productus* Randall. *Biol. Bull.*, **127**: 428:446.

Copeland, M., 1923. The chemical sense of *Palaemonetes vulgaris*. *Anat. Rec.*, **24**: 394.

Crisp, D. J., 1967. Chemoreception in cirripedes. *Biol. Bull.*, **133**: 128–140.

Daum, R. J., and C. Givens, 1963. *Potency probit analysis*. U .S. Dept. of Agriculture, Biometrical Services, Beltsville, Md., 23 pp.

Davenport, D., 1966. Analysis of behavior in symbiosis. Pages 381–429 in S. M. Hendry, Ed., *Symbiosis*. Academic Press, N. Y.

Gurin, S., and W. E. S. Carr, 1971. Chemoreception in *Nassarius obsoletus:* the role of specific stimulatory proteins. *Science*, **174**: 293–295.

HEEB, M. A., 1973. Large molecules and chemical control of feeding behavior in the starfish *Asterias forbesi, Helgolander Wiss. Meeresunters,* **24**: 425–435.

KITTREDGE, J. S., M. TERRY, AND F. T. TAKAHASHI, 1971. Sex pheromone activity of the molting hormone, crustecdysone, on male crabs. *Fish. Bull., Nat. Mar. Fish. Serv.,* **69**: 337–343.

KITTREDGE, J. S., F. T. TAKAHASHI, J. LINDSEY, AND R. LASKER, 1974. Chemical signals in the sea: marine allelochemics and evolution. *Fish. Bull., Nat. Mar. Fish. Serv.,* **72**: 1–11.

LAVERACK, M. S., 1963. Aspects of chemoreception in crustacea. *Comp. Biochem. Physiol.,* **8**: 141–151.

LAVERACK, M. S., 1964. The antennular sense organs of *Panulirus argus. Comp. Biochem. Physiol.,* **13**: 301–321.

LEVANDOWSKI, M., AND E. S. HODGSON, 1965. Amino acid and amine receptors of lobsters. *Comp. Biochem. Physiol.,* **16**: 159–161.

LINDSTEDT, K. J., 1971. Chemical control of feeding behavior. *Comp. Biochem. Physiol.,* **39A**: 553–581.

MANGUM, C. P., AND C. D. COX, 1971. Analysis of the feeding response in the onyphid polychaete *Diopatra cuprea* (Bosc). *Biol. Bull.,* **140**: 215–229.

MCLEESE, D. W., 1970. Detection of dissolved substances by the American lobster (*Homarus americanus*) and olfactory attraction between lobsters. *J. Fish. Res. Bd. Canada,* **27**: 1371–1378.

RYAN, E. R., 1966. Pheromone: evidence in a decapod crustacean. *Science,* **151**: 340–341.

SCANU, A. M., AND C. WISDOM, 1972. Serum lipoproteins structure and functions. *Ann. Rev. Biochem.,* **41**: 703–730.

WHITE, A., P. HANDLER, AND E. L. SMITH, 1964. *Principles of biochemistry.* (3rd ed.) McGraw-Hill Book Co., N. Y., 1106 pp.